Consumer and Corporate Affairs Canada | Consommation et Corporations Canada

(11) (A) No. 1 254 525

(45) ISSUED 890523

(52) CLASS 195-1.12
C.R. CL. 150-8.5

(51) INT. CL.[4] C12N 15/00, C12Q 1/68

(19) (CA) **CANADIAN PATENT** (12)

(54) Kit for Terminally Chemically Labeling DNA

(72) Brakel, Christine L.;
Stavrianopoulos, Jannis G.,
U.S.A.

(73) Granted to Enzo Biochem, Inc.
U.S.A.

(21) APPLICATION No. 450,631

(22) FILED 840327

(30) PRIORITY DATE (US) U.S.A. (484,583) 830413

No. OF CLAIMS 21 - NO DRAWING

Canada

DISTRIBUTED BY THE PATENT OFFICE, OTTAWA.
CCA-274 (11-82)

ABSTRACT OF THE DISCLOSURE

Processes and reagent kits employing terminal deoxynucleotide transferase are provided for terminally chemically labeling DNA probe molecules, such as the terminal or end labeling of DNA probes with biotinylated nucleotides, e.g. 5-alkylamino biotin-labeled dUTP.

WHAT IS CLAIMED IS:

1. A reagent kit useful for the preparation of a terminally linked or labeled DNA molecule or probe comprising in packaged combination: (1) a container of terminal deoxynucleotidyl transferase, hereinafter referred to as TdT, (2) a container of a DNase capable of creating or exposing a 3'-OH terminus of DNA, and (3) a container of chemically labeled deoxyribonucleotide capable of being introduced into DNA by said TdT.

2. A reagent kit according to Claim 1, wherein said chemically labeled deoxyribonucleotide when included in a DNA molecule does not prevent said DNA molecule from forming double-stranded DNA.

3. A reagent kit according to Claim 1, wherein said chemically labeled deoxyribonucleotide comprises a chemical label moiety capable of being detected by physical, physio-chemical, chemical or immunological techniques.

4. A reagent kit according to Claim 1, wherein said chemically labeled deoxyribonucleotide is selected from the group consisting of a biotinylated deoxyribonucleotide and a glycosylated deoxyribonucleotide.

5. A reagent kit according to Claim 4, wherein said biotinylated deoxyribonucleotide is 2'-deoxyuridine triphosphate 5-allylaminebiotin.

6. A reagent kit according to Claim 1, further comprising an aqueous $CoCl_2$ and/or MgCl solution.

7. A reagent kit according to Claim 1, further comprising a DNase dilution buffer comprising 10 mM Tris HCl of a pH of about 7.5.

8. A reagent kit according to Claim 1, further comprising a solution comprising about 1 mg/ml bovine serum albumin for stabilizing the DNase.

9. A reagent kit useful for the preparation of terminally linked or labeled DNA molecules or probes comprising as reagents in packaged combination therein: (1) a container of terminal deoxynucleotidyl transferase, hereinafter referred to as TdT, (2) a container of an enzyme capable of producing or exposing a 3'-OH terminus of DNA, and (3) a container of a chemically labeled deoxyribonucleotide capable of being introduced into DNA by said TdT.

10. A method of preparing a terminally linked or labeled DNA which comprises:

treating DNA with a DNase capable of creating or exposing a 3'-OH terminus of DNA, so that a 3'-OH terminus is created or exposed; and

contacting said treated DNA with a chemically labeled deoxyribonucleotide in the presence of TdT under conditions permitting TdT to effect terminal attachment of said chemically labeled deoxyribonucleotide to the 3'-OH position presented by said DNA or to any chemically labeled deoxyribonucleotide previously attached to said DNA.

11. A method of preparing a terminally linked or labeled DNA which comprises:

treating DNA with DNase I; and

contacting the treated DNA with a chemically labeled deoxyribonucleotide in the presence of TdT under conditions permitting TdT to effect terminal attachment of

said chemically labeled deoxyribonucleotide to the 3'-OH position or terminus presented by said DNA or to any chemically labeled deoxyribonucleotide previously attached to said DNA.

12. A terminally labeled DNA molecule or probe terminally linked or labeled with a non-radioactive chemically labeled deoxyribonucleotide, wherein said non-radioactive chemically labeled deoxyribonucleotide does not prevent said terminally labeled DNA molecule or probe or the terminally labeled portion of said terminally labeled DNA molecule or probe from forming double-stranded DNA.

13. A terminally labeled DNA molecule or probe according to Claim 12, wherein said non-radioactive chemically labeled deoxyribonucleotide is capable of being bound to a 3'-OH terminus of said DNA molecule or probe by terminal deoxynucleotidyl transferase, also referred to as TdT.

14. A terminally labeled DNA molecule or probe according to Claim 12, wherein said chemically labeled deoxyribonucleotide is capable of being detected by physical, physio-chemical, chemical or immunological techniques.

15. A terminally labeled DNA molecule or probe according to Claim 12, wherein said deoxyribonucleotide is selected from the group consisting of a biotinylated deoxyribonucleotide and a glycosylated deoxyribonucleotide.

16. A terminally labeled DNA molecule or probe according to Claim 15, wherein said biotinylated deoxyribonucleotide is 2'-deoxyuridine triphosphate 5-allylamine-biotin.

17. A terminally labeled DNA molecule or probe terminally linked or labeled with a single-stranded polydeoxyribonucleotide containing at least one non-radioactive chemically labeled deoxyribonucleotide, wherein said chemically labeled deoxyribonucleotide does not prevent said terminally labeled DNA molecule or probe or the terminally labeled portion of said terminally labeled DNA molecule or probe from forming double-stranded DNA.

18. A terminally labeled DNA molecule or probe according to Claim 17, wherein said chemically labeled deoxyribonucleotide is selected from the group consisting of a biotinylated deoxyribonucleotide and a glycosylated deoxyribonucleotide.

19. A terminally labeled DNA molecule or probe according to Claim 18, wherein said biotinylated deoxyribonucleotide is 2'-deoxyuridine triphosphate 5-allylamine-biotin.

20. A terminally labeled DNA molecule or probe terminally linked or labeled with a single-stranded polydeoxyribonucleotide containing at least one non-radioactive chemically labeled deoxyribonucleotide, wherein said chemically labeled deoxyribonucleotide comprises from about 1% up to 100% of the nucleotides in said single-stranded polydeoxyribonucleotide.

21. A terminally labeled DNA molecule or probe according to Claim 20, wherein said chemically labeled deoxyribonucleotide comprises from about 2% to about 40% of the nucleotides in said single-stranded polydeoxyribonucleotide.

KIT FOR TERMINALLY CHEMICALLY LABELING DNA

BACKGROUND OF THE INVENTION

Terminal deoxynucleotidyl transferase is known and has been employed to catalyze the polymerization of deoxynucleoside triphosphates for the elongation of polydeoxynucleotide chains or molecules (DNA). The utilization and effectiveness of terminal deoxynucleotidyl transferase is described by F. J. Bollum in the article entitled "Terminal Deoxynucleotidyl Transferase", in the publication The Enzymes; (P.D. Boyer, ed.), 3rd Ed. Vol. 10, pp. 145-171, Academic Press, New York, N.Y. (1974).

Another publication of interest with respect to terminal transferase (terminal deoxynucleotidyl transferase) and its utilization for the addition of homopolymers to the 3' ends of DNA is the article by T. Nelson and D. Brutlag entitled "Addition of Homopolymers to the 3'-Ends of Duplex DNA with Terminal Transferase", which appeared in Methods in Enzymology, Vol. 68, pp. 41-50, Academic Press (1979). Also of interest with respect to the utilization of terminal nucleotidyl transferase is the article by C. Vincent, P. Tchen, M. Cohen-Solal and P. Kourilsky entitled "Synthesis of 8-(2-4 dinitrophenyl 2-6 aminohexyl) amino-adenosine 5' triphosphate: biological properties and potential uses", which appeared in Nucleic Acids Research, Vol. 10, No. 21, published by IRL Press Limited, Oxford, England (1982). The above-identified article by Vincent et al discloses that the above-entitled compound is a substrate for calf thymus deoxynucleotidyl terminal transferase and

that this compound can be incorporated into DNA molecules by elongation from 3' ends and that the incorporated nitrophenyl group can be recognized by specific antibodies which can then be detected by anti-antibodies coupled to an enzyme. If desired, the dinitrophenyl group can be introduced into DNA after enzymatic incorporation of 8-aminohexyl adenosine 5' triphosphate and reaction with 1-fluoro-2-4-nitrobenzene.

Of additional interest is the article by P. R. Langer, A. A. Waldrop & D.C. Ward entitled "Enzymatic synthesis of biotin-labeled polynucleotides: Novel nucleic acid affinity probes", Proc. Natl. Acad. Sci., Vol. 78, No. 11, pp. 6633-6637, November 1981, which discloses certain biotinylated nucleotides capable of incorporation in double-stranded DNA and useful as substrates for a variety of DNA and RNA polymerases. It is suggested in this article that the disclosed biotin-labeled polynucleotides would be useful as affinity probes for the detection and isolation of specific DNA and RNA sequences.

Of special interest related to the practices of this ivnention is U.S. Patent 4,358,535 which issued to S. Falkow, and S. Mosley entitled "Specific DNA Probes in Diagnostic Microbiology".

It is an object of this invention to provide special terminally labeled DNA molecules useful as DNA probes.

It is another object of this invention to provide materials, reagents and techniques for the preparation of terminally chemically labeled DNA probes.

How these and other objects of this invention are accomplished will become apparent in the light of the accompanying disclosure.

SUMMARY OF THE INVENTION

The terminal chemical labeling of DNA molecules, such as DNA probes, is effected by employing terminal deoxynucleotide transferase (TdT). In the terminal chemical labeling of DNA molecules, there is employed in combination with TdT, as a substrate therefor, a chemically labeled nucleotide, such as a biotinylated nucleotide, e.g. 2'-deoxyuridine triphosphate 5-allyl-amine-biotin also identified as biotin-11-dUTP, or other biotinylated nucleotides capable of acting as a substrate for TdT for terminal attachment to the DNA. In the practices of this invention any nucleotide, preferably chemically labeled, useful as a substrate for TdT and capable of terminal attachment via TdT to the DNA being labeled is usefully employed. Although it is desirable that the nucleotides so terminally attached to the DNA comprise at least one biotinylated nucleotide, it is desirable that the nucleotides terminally attached to the DNA consist of more than 50% by number biotinylated nucleotides. However, nucleotides terminally attached to the DNA via TdT may consist of or comprise only a minor portion or percentage, in the range from about 2% to about 40% by number, biotinylated nucleotides, i.e. biotinylated deoxyribonucleotides.

DETAILED DESCRIPTION OF THE INVENTION

In accomplishment of the above, i.e. the terminal labeling of DNA probes, such as the end or terminal biotinylation of DNA probes via terminal deoxynucleotide transferase TdT, there are provided, in accordance with the practices of this invention, reagents and procedures employing the same for the *in vitro* preparation of DNA probes with high biotin content. By employing TdT a polymer with a biotin-containing analog of TTP is formed on the 3'-OH terminus of the DNA of interest. In accordance with the practices of this invention, there are provided reagents, in kit form, for the synthesis of a non-radioactive, chemically-labeled stable DNA probe. The special nucleotides making up said probe are detectable by techniques, such as the use of anti-antibodies directed to an antibody attached to the terminally linked special nucleotides attached to the DNA probe or by the use of, for example, enzyme-linked avidin or streptavidin which would attach itself to the biotinylated special nucleotides terminally attached to the DNA probes.

In a kit provided for the practices of this invention, i.e. for the terminal labeling of DNA probes employing terminal deoxynucleotide transferase, the kit would contain the following components or reagents or tubes:

1. Terminal deoxynucleotide transferase
   20 unit/ul in 100 mM KCadocylate ph 7.0; 5mM 2-mercaptoethanol; 50% v/v glycerol

2. Terminal deoxynucleotide transferase dilution buffer
   50 mM KCacodylate pH 7.0;
   5mM 2-mercaptoethanol; 1mg/ml bovine serum albumin (enzyme stabilizer grade ERT-701).

3. DNase
   0.5mg/ml in 0.1M $MgCl_2$

4. DNase I dilution buffer
   10mM Tris HCl ph 7.5
   1mg/ml bovine serum albumin (ERT-701).

5. 3.3 x terminal labeling reaction buffer
   .66M KCacodylate pH 7.0
   .0033M 2-mercaptoethanol

6. Deoxynucleotide solutions
   a. 9mM dTTP
   b. 9mM dCTP

7. Bio Probe (Bio-dUTP) Solution
   2.5mM Bio dUTP

8. $CoCl_2$
   0.01M

9. 3H dTTP (40-60 Ci/mmol)
   [methyl-3H] Thymidine-5'-triphosphate ammonium salt in 50% aqueous ethanol, 0.25 uCi./ul. (The 3H dTTP is used solely to monitor incorporation and is supplied by Amersham Corp.)

10. Control DNA, DNase treated
    0.5mg/ml in 50mM Tris HCl pH 7.4, 5mM $MgCl_2$, heat inactivated DNase. (to be used as terminal labeling control.)

A preferred procedure for the preparation of the DNA for terminal labeling employing the DNase component of a kit in accordance with this invention is as follows:

For general purposes, 3'OH termini introduced by DNase I are effective primer termini for terminal transferase. DNA is digested with DNase to a fragment size of 200-500 base pairs (or whatever size is desired). The DNase is subsequently inactivated by heat and the digested DNA is ready to be terminally labeled. Some variation in digestion will occur, and it is best to follow the general outline described here and then observe the size of the DNA on agarose (and/or acrylamide) gels.

In a plastic tube place

a. 5ug DNA in 5ul or less
b. 2.5ul 0.04M $MgCl_2$
c. H_20 to give 8.0ul

To this tube add 2.0ul of DNase freshly diluted in the following manner: 1ul DNase plus 99ul DNase dilution buffer; one microliter of this solution is then diluted with 49 ul DNase dilution buffer. After addition of DNase the tube is incubated for 2 to 10 minutes (5 minutes is generally optimal) at 37°C. and then for 5 minutes at 68°C. to inactivate the DNase.

It is important to note that if the DNA solution is dilute and contains EDTA, and is concentrated by lyophilization prior to digestion, the concentration of EDTA must be accounted for so that the $MgCl_2$ concentration is at least 5mM during digestion.

After inactivating the DNase, the DNase treated DNA is ready for terminal labeling.

After treatment of the DNA by DNase to prepare the DNA for terminal labeling, the following procedure is employed:

A. Dilution of Terminal Transferase

Terminal transferase (tube 1) should be diluted prior to each use by adding 1.5ul of TdT to 4.5 ul of TdT dilution buffer (tube 2).

B. Lyophilization of 3H dTTP

Lyophilize 8ul of 3H-dTTP in a plastic tube in which the reaction will be performed.

C. Terminal Labeling Reaction.

Upon removal of ethanol by lyophilization, the remaining reagents are added to 3H dTTP in the following order:

Tube No.	Components	Volume Per Reaction
5.	3.3x TdT Reaction Buffer	15ul
6a or 6b	9mM dTTP or 9mM dCTP	5ul
	OR	
6a + 6B	9mM dTTP + 9mM dCTP	2.5 ul 6a + 2.5ul 6b
7.	2.5mM Bio dUTP	2ul
	Experimental DNA to be terminally labeled	1ug
	OR	
	Control DNase-treated DNA	2ul

Adjust volume to 40ul with sterile distilled H_20 before adding:

1+2	Diluted TdT	5ul
8.	$CoCl_2$	5ul

Incubate the reaction at 37°C. for one hour. Chill reaction to 0°C. and determine incorporation (see below). If further incorporation is desired, continue to incubate the reaction at 37°C. Under most circumstances, sufficient incorporation should be obtained after 1-2 hours of incubation. Using the DNase introduced 3'OH primer termini, 10-30 nanomoles of total nucleotide are incorporated. Stop the reaction by heating at 65°C. for 5 minutes, or by adding 1/10 volume 0.1M EDTA.

D. Determination of Incorporated Nucleotide

1. Remove a 2 microliter aliquot into a 5 ml plastic tube to which 10 micrograms of poly rA has been added.
2. Add 1ml cold 5% (w/v) trichloroacetic acid (TCA), 25mM sodium pyrophosphate.
3. Keep tube in ice for at least 10 minutes.
4. Filter through glass fiber filters.
5. Wash filters thoroughly with 2.5% TCA, 10mM sodium pyrophosphate.
6. Dry thoroughly.
7. Add toluene-based liquid scintillation cocktail to cover the filter and count in a liquid scintillation counter.
8. To determine total radioactivity in the reaction mixture, transfer a second 2 microliter aliquot into 150 microliters directly (without filtration) on a glass fiber filter. Dry and count.

E. Calculation of nanomoles deoxynucleotide incorporated and estimation of biotinylated dUTP content:

Using the protocol described above and the reaction as described in part C, calculate the nanomoles of nucleotides incorporated by the following equation:

$$\frac{\text{Nanonomoles nucleotide incorporated}}{\text{ug of DNA}} = \frac{\text{cpm TCA precipitated X50}}{\text{cpm total}}$$

To estimate the content of the biotinylated dUTP per ug of probe DNA, multiply the total incorporated nucleotides by 0.10 (the fraction of Bio-dUTP in reaction).

The amount of Bio dUTP per ug of DNA should be from 1-3 nanomoles.

F. Recovery of Biotinylated DNA:

1. Load "stopped" terminal labeling reaction mixture on a Sephadex* G-50 column (approx. 3.5ml) equilibrated with 10mM Tris-HCl (pH 7.5), 1mM EDTA.
2. Collect 5 drops per fraction. Count a 2 microliter aliquot of each fraction by liquid scintillation counting.
3. Pool fractions in the first peak which contain the biotinylated DNA. Discard the second peak which contains nucleotides.
4. Store at 4°C, or -20°C.

Alternatively, DNA may be separated from unincorporated nucleotides using the "spin-column" protocol described below:

1. Pre-swell Sephadex G-50 in 10mM Tris-HCl (pH 7.5), 1mM EDTA and pipette into a 1ml tuberculin syringe that has been plugged with siliconized glass wool. Allow to settle by gravity. Fill the syringe to the top (1.2ml) with resin.

* Sephadex is a trademark.

2. Place the filled syringe through a hole cut into the cap of 15 ml plastic conical centrifuge tube. Place a pad of paper towel or kimwipe in the bottom of the conical centrifuge tube and insert a 1.5ml Eppendorf tube. Place the cap with the fitted syringe into the conical centrifuge tube so that the tip of the syringe is fitted into the opening of the Eppendorf tube.

3. Spin the tube-syringe assembly at low speed in a bench top centrifuge for 3-4 minutes. After centrifugation the packed bed volume in the syringe should be between 0.85 and 0.9ml. 4. Remove the syringe and then remove the liquid in the Eppendorf by pipet.

5. Reinsert the syringe into the hole and pipet 50 microliters of the terminal labeling reaction onto the top of the resin bed. The syringe is then recentrifuged for 3-4 minutes at the same speed as initially used to make the packed column.

6. The liquid retained in the Eppendorf contains the DNA probe free of unincorporated nucleotides in a volume equal to or slightly less than that applied (50 microliters). The volume of the sample is adjusted to give a final probe concentration of 20 microgram/ml.

As another alternative, DNA can be isolated by ethanol precipitation by the following procedure:

1. Following end labeling, add an equal volume of 4M ammonium acetate and 20 ug carrier nucleic acid (20ul) to the stopped reaction. Mix and then add 2 volumes of ice cold ethanol.

2. Precipitate the DNA fragments by chilling in a dry ice-ethanol bath for 5 minutes. Centrifuge at 12,000 x g for 5 minutes in cold to pellet the DNA. Remove the supernatant carefully with a Pasteur pipe and discard. 3. Add 250 ul of 0.3M sodium acetate to solubilize the DNA pellet. Add 750ul cold ethanol: chill, centrifuge and remove supernatant as described in Step. 2.
4. Repeat step 1.
5. Gently cover the pellet, containing 3' end labeled DNA fragments, with lml ethanol. Carefully remove the supernatant and dry the pellet under vacuum for several minutes.
6. Resuspend in desired buffer and determine recovery.

Phenol extraction of biotinylated DNA samples should be avoided because of extraction into the phenol layer or retention at the phenol-water interface.

In the practices of this invention it is preferred to employ as the chemically labeled nucleotide for the terminal labeling of DNA, the biotinylated nucleotide 2-deoxyuridine triphosphate 5-allylamine-biotin referred to hereinabove as biotin-11-dUTP or bio-dUTP. The amount of the chemically labeled nucleotide employed for the terminal labeling of the DNA is usually about 10% of the total deoxynucleotide content so employed. The percentage bio-dUTP, if desired, can be increased up to 100% or decreased to about 1% to produce terminally labeled DNA probes of higher or lower specific activity, respectively. Usually, 10% bio-dUTP content is suitable for most terminal labeling purposes.

As indicated hereinabove, it is to be emphasized that bio-dUTP may be replaced by any of the chemically labeled specially modified deoxynucleotides described or referred to hereinabove.

With respect to the utilization of ^{3}H dTTP, this component can be replaced with any other radiolabeled deoxynucleoside triphosphate or, if desired, the radiolabeled nucleoside can be completely omitted. It is pointed out also that the use of more terminal transferase per reaction results in a more rapid synthesis and, conversely, the use of less terminal transferase reduces the rate of reaction or synthesis of the terminally labeled DNA.

It has been found that the concentrations of the components or constituents in the reaction have marked effect on the resulting terminally labeled probe. For example, adding more or less deoxynucleotide, with time, the terminal tranferase will polymerize up to 90% of the added dCTP or dTTP. Accordingly, as one increases

the amount of the added nucleotide longer and longer "tails" are formed. Also, if the concentration of DNA to be labeled is varied, the number of moles of nucleotide incorporated changes. For example, as the amount of DNA in the reaction increases a plateau region is reached wherein the total nucleotide incorporation is constant but absolute tail length and numbers of the incorporated chemically labeled nucleotide, e.g. bio-dUTP per molecule of DNA decreases. Further, the concentration of or metal ion content, e.g. Co^{++} and/or Mg^{++} influences the terminal labeling operation and resulting terminally labeled DNA.

It is mentioned hereinabove that the DNA terminally labeled with the chemically labeled or modified nucleotides can be detected by non-radioactive techniques, such as by means of anti-antibodies directed to the terminally linked special nucleotides or by the use of detectors, such as avidin or streptavidin, or enzyme-linked avidin or enzyme linked streptavidin, which would then attach themselves to the biotin component or moiety of the chemically labeled nucleotide. If desired, the terminally linked special nucleotides could be detected by means of radioactive techniques, such as the use of radioactive avidin, to detect or locate terminally linked biotinylated nucleotide in accordance with the practices of this invention.

As mentioned hereinabove, it is preferred in the practices of this invention to employ as the terminally linked nucleotides or as substrate for the terminal transferase biotinylated nucleotides, either biotin or iminobiotin labeled nucleotides. See the disclosures hereinabove and the nucleotides disclosed in the above-identified Langer et al publication P.N.A.S. Vol. 78, No. 11, pp. 6633-6637 (1981). Of special interest

as the chemically labeled nucleotides are the glycosylated nucleotides. These special nucleotides, which are also capable of being incorporated into double-stranded DNA and employed, as disclosed herein, as substrate for terminal transferase or for terminal linking to DNA, are capable, when terminally linked to DNA, of being readily detected by means of a lectin, such as Concanavalin A. The determination or detection of such glycosylated terminally linked deoxynucleotides can be carried out by employing a radioactive labeled lectin or by means of an antibody or anti-antibody or by means of enzyme linked lectin in the manner mentioned hereinabove with respect to the detection or determination of biotin-labeled terminally linked nucleotides.

The practices of this invention, as indicated hereinabove, are applicable to the preparation of terminally labeled single-stranded and double-stranded DNA.

Also, as indicated hereinabove, the chemically labeled terminal portion or tails of the DNA molecules or probes in accordance with this invention can be detected by a variety of techniques, such as radioactive detection techniques, enzyme based techniques and immunoassay or antibody based techniques. For example, when the chemically labeled nucleotides making up the tail portion of the DNA molecule contains a biotin moiety, the presence or location of the chemically labeled tail (the biotin moiety) could be detected employing a radioactive avidin or streptavidin or avidin linked to a

biotinylated enzyme, such as a complex made up of avidin-biotin-alkaline phosphatase, or streptavidin-biotin-horseradish peroxidase. After attachment of the complex to the biotin moiety of the chemically labeled nucleotide, its presence, for example, would be elicited by a suitable color response or change by the action of the enzyme horseradish peroxidase or alkaline phosphatase on a suitable color generating or color changing substrate. Further, also, the presence of a chemically labeling moiety, such as biotin, in the chemically labeled nucleotide could be elicited or detected by contact with an antibody, such as goat antibody, to biotin and then by contact with a rabbit anti-goat antibody, which would be a linked to an enzyme. A similar approach would be applicable to the detection of a chemically labeled nucleotide wherein the labeled nucleotide making up the tail is glycosylated or includes a glycosidic linkage. Such glycosylated nucleotides could be detected by means of, for example, radioactive lectin, e.g. radioactive Concanavalin A, or by means of other chemicals or antibodies responsive to lectin or enzymes linked to a lectin. Lectin would elicit the presence of a glycosylated nucleotide in accordance with this invention since lectin readily attaches itself to a glycosyl moiety, like avidin readily attaches itself to a biotin moiety. Enzyme-based techniques applicable to the practices of this invention for the detection of the chemically labeled nucleotides are described in copending, coassigned Canadian Patent Application Serial No. 445,896 filed January 23, 1984. Like the DNA probes described in patent application Serial No. 445,896 the specially

terminally labeled DNA or RNA molecules described herein are also capable of being fixed to a transparent substrate, such as glass, and utilized in the manner described in patent application Serial No. 445,896

Although in the practices of this invention described herein the enzyme DNase has been employed for the preparation of the DNA to be terminally labeled, other enzymes are also usefully employed. For example, restriction endonucleases can be used for the production of 3'-OH termini for subsequent terminal chemical labeling with terminal transferase in accordance with this practices of this invention. Restriction endonuclease Pst 1 which produces protruding 3'-OH termini and restriction endonuclease Bam HI which produces recessed 3'-OH termini have been employed for this purpose. Successful labeling required an extended incubation with an approximate 2 to 4-fold increase in the amount of terminal transferase in each reaction. Lambda exonuclease, which exposes the recessed 3'-OH termini produced by most restriction endonucleases further enhances the effectiveness of these DNAs as primers for terminal transferase. Flush 3'-OH termini, produced by enzymes such as Hae III, are also suitable. However, restriction enzyme digested DNA must be purified by phenol extraction and ethanol precipitation prior to terminal labeling. For this reason DNase digestion is preferred. Also, randomly sheared DNA, sheared by sonication or other means, can also be terminally labeled. However, there is little or no advantage of this technique over the use of DNase to generate primer termini.

As will be apparent to those skilled in the art in the light of the foregoing disclosure, many modifications, alterations and substituions are possible in the practices of this invention without departing from the spirit or scope thereof.